Anti-Smad2 antibody [EP784Y] ab40855

Product datasheet

Overview

Product name: Anti-Smad2 antibody [EP784Y]
Description: Rabbit monoclonal [EP784Y] to Smad2
Host species: Rabbit
Specificity: This antibody is specific for MH 1 domain of Smad2.
Tested applications: Suitable for: IHC-P, IP, ICC/IF, WB, Flow Cyt
Species reactivity: Reacts with: Rat, Human
Immunogen: Synthetic peptide within Human Smad2 aa 50-150. The exact sequence is proprietary.
Positive control: Jurkat cell lysate and human prostate carcinoma tissue. IP: HeLa
General notes: The rat recommendation is based on the WB results. This antibody may not be suitable for IHC with rat samples.

We recommend using this antibody on human tissues/cell lines. For mouse tissues/cell lines we suggest the use of our mouse monoclonal antibody to ribosomal protein S6 kinase, alpha 1 [EP328Y].

Properties

Form: Liquid
Storage instructions: Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer:
- pH: 7.20
- Preservative: 0.01% Sodium azide
- Constituents: 40% Glycerol, 0.05% BSA, 59% PBS

Purity: Protein A purified

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### Function
Receptor-regulated SMAD (R-SMAD) that is an intracellular signal transducer and transcriptional modulator activated by TGF-beta (transforming growth factor) and activin type 1 receptor kinases. Binds the TRE element in the promoter region of many genes that are regulated by TGF-beta and, on formation of the SMAD2/SMAD4 complex, activates transcription. May act as a tumor suppressor in colorectal carcinoma.

### Tissue specificity
Expressed at high levels in skeletal muscle, heart and placenta.

### Sequence similarities
Belongs to the dwarfin/SMAD family.
Contains 1 MH1 (MAD homology 1) domain.
Contains 1 MH2 (MAD homology 2) domain.

### Post-translational modifications
Phosphorylated on one or several of Thr-220, Ser-245, Ser-250, and Ser-255. In response to TGF-beta, phosphorylated on Ser-465/467 by TGF-beta and activin type 1 receptor kinases. Able to interact with SMURF2 when phosphorylated on Ser-465/467, recruiting other proteins, such as SNON, for degradation. In response to decorin, the naturally occurring inhibitor of TGF-beta signaling, phosphorylated on Ser-240 by CaMK2. Phosphorylated by MAPK3 upon EGF stimulation; which increases transcriptional activity and stability, and is blocked by calmodulin. In response to TGF-beta, ubiquitinated by NEDD4L; which promotes its degradation. Acetylated on Lys-19 by coactivators in response to TGF-beta signaling, which increases transcriptional activity. Isoform short: Acetylation increases DNA binding activity in vitro and enhances its association with target promoters in vivo. Acetylation in the nucleus by EP300 is enhanced by TGF-beta.

### Cellular localization

### Applications
Our **Abpromise guarantee** covers the use of **ab40855** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
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<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tr>
<td>IHC-P</td>
<td>1/50</td>
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<tr>
<td>IP</td>
<td>1/20 - 1/50</td>
<td></td>
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<tr>
<td>ICC/IF</td>
<td>1/100 - 1/250</td>
<td></td>
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<tr>
<td>WB</td>
<td>1/2000 - 1/10000. Detects a band of approximately 55 kDa (predicted molecular weight: 58 kDa).</td>
<td></td>
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<tr>
<td>Flow Cyt</td>
<td>1/20 - 1/100.</td>
<td><strong>ab172730</strong> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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</table>

**Target**

**Function**
Receptor-regulated SMAD (R-SMAD) that is an intracellular signal transducer and transcriptional modulator activated by TGF-beta (transforming growth factor) and activin type 1 receptor kinases. Binds the TRE element in the promoter region of many genes that are regulated by TGF-beta and, on formation of the SMAD2/SMAD4 complex, activates transcription. May act as a tumor suppressor in colorectal carcinoma.

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**Cellular localization**
Western blot - Anti-Smad2 antibody [EP784Y] (ab40855)

**All lanes**: Anti-Smad2 antibody [EP784Y] (ab40855) at 1/1000 dilution

**Lane 1**: Wild-type HeLa whole cell lysate

**Lane 2**: Smad2 knockout HeLa whole cell lysate

**Lane 3**: A549 whole cell lysate

Lysates/proteins at 20 µg per lane.

**Predicted band size**: 58 kDa

**Lanes 1 - 3**: Merged signal (red and green). Green - ab40855 observed at 58 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab40855 was shown to specifically react with Smad2 in wild-type HeLa cells as signal was lost in Smad2 knockout cells. Wild-type and SMAD2 knockout samples were subjected to SDS-PAGE. Ab40855 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/10000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.
All lanes: Anti-Smad2 antibody [EP784Y] (ab40855) at 1/2000 dilution

Lane 1: A-673 (Human muscle Ewing's Sarcoma cell line) whole cell lysate
Lane 2: HUVEC (Human umbilical vein endothelial cell line) whole cell lysate
Lane 3: C6 (Rat glial tumor cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 58 kDa

Diluting and blocking buffer: 5% NFDM/TBST

ab40855 staining Smad2 in HeLa (human cervix adenocarcinoma) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/100. A goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) was used as the secondary antibody at a dilution of 1/1000. ab195889 was used as a counterstain for primary antibody ab40855 at 1/1000. DAPI was used as a nuclear counterstain and PBS as a negative control.
**Western blot - Anti-Smad2 antibody [EP784Y] (ab40855)**

**All lanes**: Anti-Smad2 antibody [EP784Y] (ab40855) at 1/10000 dilution

**Lane 1**: Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

**Lanes 2-3**: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 20000 µg (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

**Predicted band size**: 58 kDa

Blocking and diluting buffer: 5% NFDM/TBST

ab40855 staining Smad2 in human bladder carcinoma tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/50. A ImmunoHistoProbe one step HRP Polymer was used as a secondary antibody, ready to use.

**Negative control 1**: PBS in place of primary antibody.
Immunoprecipitation - Anti-Smad2 antibody [EP784Y] (ab40855)

**ab40855 (purified) at 1/20 immunoprecipitating EGFR in HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate.**

Lane 1 (input): HeLa whole cell lysate (10µg).

Lane 2 (+): ab40855 + HeLa whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab40855 in HeLa whole cell lysate.

For western blotting, ab131366 VeriBlot for IP (HRP) was used for detection (1/1000).

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.

Flow Cytometry - Anti-Smad2 antibody [EP784Y] (ab40855)

**ab40855 staining Smad2 in the human cell line HeLa (Human epithelial cell line from cervix adenocarcinoma) by flow cytometry.**

Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/20. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.

**Isoytype control: Rabbit monoclonal IgG (Black)**

**Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)**

Immunocytochemistry/ Immunofluorescence - Anti-Smad2 antibody [EP784Y] (ab40855)

**Immunofluorescence staining of HeLa cells with purified ab40855 at a working dilution of 1/500, counter-stained with DAPI. The secondary antibody was an Alexa Fluor® 488 conjugated goat anti-rabbit (ab150077), used at a dilution of 1/1000. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative control is shown in bottom right hand panel - for the negative control, PBS was used instead of the primary antibody.**
Overlay histogram showing PC3 cells stained with ab40855 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab40855, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1μg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

Anti-Smad2 antibody [EP784Y] (ab40855) at 1/50000 dilution + Jurkat cell lysate

**Predicted band size:** 58 kDa  
**Observed band size:** 58 kDa

ab40855 at a 1:100 dilution staining Smad2 in human prostate carcinoma tissue.

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